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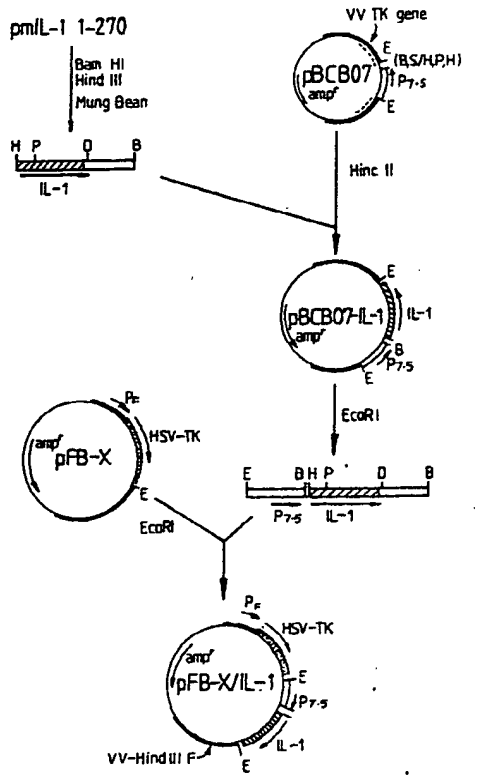
(54) Title: RECOMBINANT VACCINE

(57) Abstract

A recombinant vaccine comprises a vaccine vector which incorporates a first nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide, together with a second nucleotide sequence capable of being expressed as all or a part of a lymphokine effective in enhancing the immune response to the antigenic polypeptide. The vaccine vectors include poxvirus, herpes virus or adenovirus, and the lymphokine may be an interleukin or gamma-interferon. The vaccine vector may express an antigenic polypeptide which is foreign to the host vector.



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"RECOMBINANT VACCINE"

This invention concerns new vaccines developed using recombinant DNA technology to provide useful immune responses in circumstances where traditional vaccines may not be sufficiently effective.

Many existing live or killed vaccines are not without disadvantages, often significant, in respect of, for example, high production costs, poor response, low response to poorly immunogenic antigens, instability and a requirement for adjuvants. Furthermore, alternative vaccine preparations based on agents such as purified proteins or synthetic peptide antigens frequently offer only poor protection. In response to these problems, attention has turned to the development of vaccines in which recombinant DNA methods have been used to introduce antigens to which immunity is required, into carrier viruses such as vaccinia.

The advantage of the recombinant DNA approach is that an infectious recombinant virus simultaneously synthesizes the foreign polypeptide and viral antigen, which can then be delivered to a host immune system as a superficial skin lesion. Vaccinia viruses have, for example, been modified for expression of the genes for hepatitis B, human immunodeficiency virus, influenza and malaria antigens; the construction of recombinant viruses carrying other antigens of medical or veterinary importance is under investigation.

In some instances, however, the immune response of recombinant vaccines may be of limited nature and magnitude. Thus, while peripheral immunization with vaccinia-influenza recombinants provides good protection against lower respiratory tract infection, it fails to induce immunity in the upper respiratory tract. On the other hand, peripheral immunization with recombinant vaccines may prove ineffective when local rather than systemic immunity is required, as in say the gastro-intestinal tract.

There have been various attempts to remedy these deficiencies, including expression of vaccine antigens through viruses having stronger promoters, such as poxvirus, but to date these have not met with significant success. The present invention provides an effective means for enhancing the immune response to the specific foreign antigenic polypeptides of recombinant vaccines.

The immune system is regulated in part by molecules, known as lymphokines, which are released by lymphocytes and help or modify the functions of other classes of lymphocytes. The present invention

is based on a recognition that the expression of appropriate lymphokines from recombinant bacterial or viral vaccines can boost and/or modify the immune response to viral, bacterial or co-expressed foreign antigenic polypeptides.

Accordingly, in its broadest aspect, this invention provides a recombinant vaccine comprising a vaccine vector which incorporates a first nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide, together with a second nucleotide sequence capable of being expressed as all or a part of a lymphokine effective in enhancing the immune response to the antigenic polypeptide.

In accordance with one embodiment of this invention, the first nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide may be a "native" sequence of the host vector itself, for example vaccinia or herpes virus. In this embodiment, administration of the vaccine of this invention provides augmentation and/or selective induction of the immune response to the "native" antigenic polypeptide.

In another embodiment, the vaccine vector incorporates a nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide which is foreign to the host vector. In this embodiment, administration of the vaccine to an individual will result in augmentation and/or selective induction of the immune response of the individual to both antigenic polypeptide of the host vector and to co-expressed foreign antigenic polypeptide.

In another aspect, the present invention provides a method for producing an immune response in

a human or animal, in particular an immunodeficient or immunosuppressed human or animal, which comprises the step of administering to the human or animal a recombinant vaccine as broadly described above.

It will be appreciated from the broad description set out above that the present invention has particular application in the augmentation of immune responses in immunodeficient or immunosuppressed individuals. In one particularly important aspect of this invention, there is provided a recombinant vaccine for use in the treatment of immunodeficient or immunosuppressed individuals infected with the human immunodeficiency virus (HIV), which comprises a vaccine vector which incorporates a nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide derived from the human immunodeficiency virus (HIV), together with a second nucleotide sequence capable of being expressed as all or a portion of a lymphokine effective in enhancing the immune response of the individual to the HIV antigenic polypeptide.

The lymphokines which may be expressed in vaccines according to this invention include those designated interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4) and γ-interferon (γ-IFN).

Interleukin-1 is a peptide hormone largely produced by activated macrophages. IL-1 modulates the proliferation, maturation and functional activation of a broad spectrum of cell types (1-3) and plays a major role in the initiation and amplification of immune and inflammatory responses through its action on these diverse cell populations (4). The gene for murine (5) and human (6) IL-1 has

been cloned and expressed in E.coli.

Interleukin-2 is a lymphokine produced by helper T cells and is active in controlling the magnitude and type of the immune response (7). Other functions have also been ascribed to IL-2 including the activation of NK cells (8) and the stimulation of cell division in large granular lymphocytes and B cells (9). Numerous studies in mice and humans have demonstrated that deficient immune responsiveness both in vivo and in vitro can be augmented by IL-2. For example, exogenous IL-2 can restore the immune response in cyclophosphamide-induced immunosuppressed mice (10) and athymic (nude) mice (11). Furthermore, IL-2 can restore responsiveness of lymphocytes from patients with various immunodeficiency states such as leprosy and cancer (12). IL-2 has also been used for the treatment of cancer (13). The gene for immune (14) and human (15) IL-2 has been cloned and sequenced.

Interleukin-3 is a hormone-like glycoprotein produced by lectin or antigen activated T lymphocytes and possibly other cells within the bone marrow. The hormone stimulates the growth and differentiation of haematopoietic progenitor cells and multipotential stem cells and has been described under a variety of names, among them multi-colony stimulating factor, and haematopoietic growth factor (16). The gene for mouse IL-3 has been cloned and sequenced (17).

Interleukin-4 is a T cell derived factor that acts as an induction factor on resting B cells, as a B cell differentiation factor and as a B cell growth factor (18). The factor also stimulates T cells and acts as a mast cell growth factor (18). The gene for murine (19) and human (20) IL-4 has been

isolated and sequenced.

γ-interferon is also a T cell derived molecule which has profound effects on the immune response. The molecule promotes the production of immunoglobulin by activated B cells stimulated with interleukin-2. γ-interferon also increases the expression of histocompatibility antigens on cells which associate with viral antigens to stimulate cytotoxic T cells. The gene for human γ-interferon has been isolated and sequenced (21).

The co-expression of a lymphokine such as IL-2 and an antigenic polypeptide by a recombinant vaccine (such as a recombinant virus vaccine) ensures that they are produced together by the same infected cells in a very localized area. This can be expected to lead to an elevation and acceleration of response to the virus vector component of the vaccine, e.g. vaccinia virus, with attendant benefits such as a reduction in the risks of complication associated with the use of vaccinia virus in normal individuals, and to those unidentifiable individuals who react adversely to vaccinia virus. Also, where there are immunological defects, as in the case of patients suffering from AIDS, leprosy or cytomegalovirus infection, co-expression of lymphokine could be instrumental in overcoming the defects to allow a normal response to the antigenic polypeptide and/or vector virus.

Furthermore, it is anticipated that the present invention will prevent or at least minimise the complications such as generalised vaccinia that can occur when vaccine is administered inadvertently to immunodeficient r cipients (32).

Further, cancer patients often show a

negligible or poor immunological response to the cancer antigens. It may be possible to enhance those responses to useful levels by taking cancer cells from the hosts, infecting them with, say, vaccinia virus/IL-2 recominants, and returning them to the patient. To guard against generalized vaccinia infection or spread of the cancer cells it may, of course, be advisable to inactivate the recombinant-infected cancer cells prior to return to the patient.

Other lymphokines (e.g. IL-1, IL-3, IL-4 and γ-IFN), are involved in the control and augmentation of responses in other parts of the immune system including granulocyte-macrophage lineage, eosinophil differentation and mucosal immunity. Construction of co-expressive vaccines will enable advantage to be taken of these specific modes of activity. Thus, as they are believed to have a role in the generation of protective responses at mucosal surfaces, such as in the gut, which promote expulsion of and immunity to parasites, a vaccine co-expressing IL-3 (or other lymphokine) with a helminth or other parasite antigenic polypeptide would be expected to give rise to enhanced immunity compared to that from the parasite antigen alone. Whilst a specific example of co-expression of the influenza haemagglutinin (HA) is described in detail herein, it will be appreciated that the present invention may be applied for the co-expression of other antigens including hepatitis virus, herpes simplex virus, Epstein-Barr virus and human immunodeficiency virus antigens, as well as malaria antigens.

As previously described, vaccinia virus has

been used as a vaccine vector to deliver antigens of unrelated infectious agents such as hepatitis B virus (22) and human immunodeficiency virus (23). The expression of an inserted gene in vaccinia virus requires that the gene be placed next to a vaccinia promoter. The promoter usually used is designated p7.5 (24). This chimeric gene is then placed next to a DNA fragment of vaccinia virus taken from a non-essential region of the virus. Insertion into infectious virus is by homologous recombination in which a marker rescue is used to select for virus recombinants. By way of example, the marker rescue can be either selection for thymidine kinase negative (TK^-) virus in which the foreign gene has been inserted and thereby inactivating the TK gene; or by selecting for TK^+ virus in which the foreign gene is flanked by the herpes simplex TK gene. The latter is generally used to construct double recominants that is, viruses expressing two foreign genes.

The expression of lymphokine genes in vaccinia virus may be detailed as two stages; the first is to create a plasmid in which the lymphokine is under the control of a vaccinia promoter 7.5 and downstream from a thymidine kinase (HSV) gene. This plasmid is then used to transfect TK^- cells previously infected with a TK^- vaccinia virus expressing another foreign gene. TK^+ recombinant virus is then selected by culturing cells in the presence of methotrexate.

Although this invention has primarily been described with reference to vaccinia virus as the vaccine vector, it is to be understood that the inventive concept resides in co-expression of an antigenic polypeptide and lymphokine, and this

concept may be realized using other vaccine vectors, such as other poxvirus, herpes virus, adenovirus or bacteria.

It is also to be understood that the invention is not limited by application to man or other species specifically mentioned herein, but may find application in a wide range of animal species.

Methods for construction and testing of recombinant vaccines according to this invention will be well known to those skilled in the art, however, for better understanding of the invention some typical techniques will now be described. Standard procedures for endonuclease digestion, ligation and electrophoresis were carried out in accordance with the manufacturer's or supplier's instructions. Standard techniques are not described in detail and will be well understood by persons skilled in the art.

EXAMPLE 1

Plasmids containing IL-1, IL-2, IL-3, IL-4 and γ-interferon are shown in Figures 1-5. Plasmids pmIL-11.270, pcD-JL-3, pcD-HuIL-2, pcD-IL-4 and pcD-HuγINF were obtained from DNAX Research Institute. The excised coding sequence is shown as the hatched bar. It is necessary to use different restriction endonucleases to create suitable termini for insertion into plasmid; these are detailed in the respective diagrams. pBCBO7 (25) contains the vaccinia 7.5K promoter, pFB-X (27) contains the HSV TK coding sequence (stippled) inserted at a BamHI site downstream from a promoter in the vaccinia HindIII region. The recombinant plasmids pFB-X/IL1, pFB-X/IL2, pFB-X/IL3, pFB-X/IL4, pRB-X/γ-interferon contain HSV-TK and lymphokine genes 3' to different vaccinia virus promoters and with flanking sequences

derived from the HindIII F fragment of vaccinia virus. The orientations of genes and promoters are shown with arrows, vaccinia virus sequences with solid lines and plasmid DNA by thin lines. The pFB-plasmids with inserted lymphokine genes are used to transfect 143B (TK^-) cells previously infected with a TK^- vaccinia virus according to conditions previously described (24). The site of insertion of HSV TK and lymphokine coding sequences and transposed vaccinia promotors in the vaccinia virus genome are shown in Figure 6. Vaccinia-virus WR strain HindIII restriction fragments are shown in the top line. Lower lines show in expanded form the DNA configurations at insertion sites in the HindIII J and F fragments. Orientations of coding sequences and promotor sequences are shown with arrows.

EXAMPLE 2

This example describes the construction of a recombinant vaccinia virus (VV) expressing murine IL2 and the effect of the lymphokine on virus growth and immunogenicity.

Figure 6 shows:

(a) Genomic configuration of VV recombinants. A HindIII map of VV WR strain is shown with insertion points at EcoRI (E) and BamHI (B) sites in the J and F fragments respectively. Arrows indicate orientations of VV TK gene, VV promoters and inserted influenza HA, HSV TK and murine IL2 coding sequences.

(b) Time course of IL2 production by VV-HA IL2-infected human 143B cells. IL2 activity in VV-HA-IL2-infected supernatants, circles and solid line;p VV-HA or uninf cted cell supernatants, crosses and dotted line.

Figure 7 shows the growth of vaccinia virus recombinants in the foot pads of athymic Swiss outbred nude mice (a) and euthymic CBA/H mice (b). $2x10^7$ PFU of VV-HA (triangles), VV-HA-TK (open circles) or VV-HA-IL2 (closed circles) were injected subcutaneously in 20µl into hind foot pads which were assayed for infectious virus on 143B cells on the indicated days. Points represent the titres of infectious virus present in individual mice.

As shown schematically in Figure 6a, cDNA encoding murine IL2 (14) was inserted into the HindIII F region of a VV recombinant, VV-HA (26), which already expressed the influenza haemagglutinin (HA). The IL2 recombinant virus, VV-HA-IL2, coexpressed HA and IL2 using the same VV 7.5Kd promoter but from separate sites in the viral genome. Since the herpes simplex virus (HSV) thymidine kinase (TK) gene was used as a selectable marker for virus construction a control virus VV-HA-TK, expressing HSV TK but not IL2 was constructed. Significant levels of biologically active IL2 were detected in supernatants from human 143B cells infected with VV-HA-IL2 within 4 hours and reached maximum activity around 12 hours (Figure 6b).

Athymic nude mice were inoculated into the right hind footpad with VV-HA-IL2 or control virus (VV-HA-TK). VV-HA-IL2 induced a mild swelling in the foot which resolved after several days; in contrast VV-HA-TK produced a severe necrotic lesion that remained unresolved for 30 days. After this time, high titres of virus ($6x10^5$-$1.5x10^7$PFU) were recovered from the feet of the VV-HA-TK inoculated mice but not from mice given VV-HA-IL2. This suggested that the IL2 produced by the recombinant

virus enabled immunodeficient mice to control the virus infection. The kinetics of viral clearance from the feet of CBA/H mice were not significantly different for VV-HA-TK and VV-HA-IL2 (Figure 7a). However, in nude mice, although titres of both VV-HA-TK and VV-HA-IL2 were high at day 3, indicating comparable rates of replication, VV-HA-IL2 was cleared by day 15 when no virus was detected in the feet. Titres of VV-HA-TK still remained high at day 15 (Figure 7b). Furthermore, when nude mice were injected intravenously (i.v.) with 10^6 PFU of VV-HA-IL2 or VV-HA-TK, mice given VV-HA-IL2 appeared unaffected by the virus whereas all mice given VV-HA-TK were moribund by day 15. Consistent with this result infectious virus was recovered from spleens and lungs of VV-HA-TK- but not VV-HA-IL2-infected mice (Table 1).

TABLE 1. Vaccinia Virus recovered from Lungs and Spleen

Mouse Strain	Organ	Log.10 VV-HA-TK	Log.10 VV-HA-IL2
CBA/H	Lung	<2.0	<2.0
	Spleen	<2.0	<2.0
Outbred Nude	Lung	4.91±0.27	<2.0
	Spleen	3.64±0.11	<2.0

Lungs and spleens were collected from 3-4 mice/group 11 days post i.v. inoculation with 10^6 PFU of the indicated virus.

Methods

(a) Murine IL-2 cDNA was subcloned from pcD-IL-2, (14) generously provided by Dr K.I. Arai, DNAX Research Institute, Palo Alto, California. VV-HA-IL1 and VV-HA-TK were constructed by insertion of the HSV TK gene plus a chimeric promoter-IL2 fragment or alone, into the HindIII F region of the recombinant VV-HA (previously described as VV-PR8-HA6) (26) using plasmids as described in Example 1 and appropriate selection protocols (27, 28).

(b) 143B cells $2x10^6$ were infected with VV-HA-IL2 at 5 PFU/cell. The supernatants (1.5ml), harvested at the time points indicated, were assayed for IL2 activity using CTLL-2 cells (29) and the colourimetric method for cell growth of Mosmann (30). The results are presented as the log dilution of supernate producing 50% of maximum proliferation in the cell cultures.

EXAMPLE 3

A vaccinia virus expressing IL-3 was constructed as shown in Figure 2, using the methods described in Example 1.

Irradiated mice (650 Rads) injected with vaccinia virus (10^6PFU intravenously) expressing IL-3 (VV-IL-3) show a reconstituted haemotoporetic system within seven days. Spleen cell counts are given below:

	Spleen Cell Number	
	VV-IL-3	NIL
4 days	3×10^7	5×10^6
7 days	2×10^8	2×10^6
10 days	1×10^8	3×10^6

In addition, the injection of vaccinia virus expressing IL-3 (VV-IL-3) protects mice against the lethal effects of irradiation, as follows:

	Deaths	
Irradiation Dose	VV-IL-3	NIL
950 Rads	0/6	6/6

EXAMPLE 4

This example describes the construction of a recombinant adenovirus expressing interleukin genes.

The starting virus for the adenovirus construct is adenovirus type 5 deletion mutant dl 327 that lacks the Xba fragment from 78.5 map units to 84.7 map units in early region 3 (31). This deletion mutant allows the insertion of DNA without exceeding the amount of DNA that can be included in the virus particle. The removal of the E3 region also prevents production of a virus protein that complexes with the major histocompatibility heavy chain protein and reduces the cell-mediated immune response to the virus. The Bam fragment from 60 map units to the right hand end of the viral DNA is cloned in plasmid. The plasmid DNA is cut downstream of the E3 promoter with a suitable restriction enzyme and the interleukin gene inserted in place of the original E3 gene, under the control of the natural E3 promoter. The resulting plasmid containing the interleukin gene in the 60 to 100 map unit fragment of dl 327 is cut with the appropriate restriction enzyme to separate viral and plasmid DNA and transfected into cells together with the overlapping EcoRl A fragment (0 to 76 map units) of wild type virus. Recombination between the two overlapping DNA fragments will reconstitute viable adenovirus in which the E3 gene

is replaced by the interleukin gene.

EXAMPLE 5

Figure 8 outlines the construction of human immunodeficiency virus IL-2 recombinant vaccinia virus in accordance with the present invention. pFB-X/IL-2 is constructed as shown using the methods described in Example 1. The construction of pTG1125 is as previously described (35).

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CLAIMS:

1. A recombinant vaccine comprising a vaccine vector which incorporates a first nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide, together with a second nucleotide sequence capable of being expressed as all or a part of a lymphokine effective in enhancing the immune response to the antigenic polypeptide.

2. A recombinant vaccine according to claim 1, wherein said vaccine vector incorporates a nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide which is foreign to the host vector.

3. A recombinant vaccine according to claim 1 or claim 2, wherein said vaccine vector is a virus.

4. A recombinant vaccine according to claim 3, wherein said virus is a poxvirus, herpes virus or adenovirus.

5. A recombinant vaccine according to claim 4, wherein said virus is vaccinia virus.

6. A recombinant vaccine according to any one of claims 1 to 5, wherein said lymphokine is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4 and γ-interferon.

7. A method for the production of a recombinant vaccine according to any one of claims 1 to 6, which

comprises the step of inserting into a vaccine vector a nucleotide sequence capable of being expressed as all or a part of a lymphokine.

8. A method according to claim 7, which comprises the further step of inserting into the vaccine vector a nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide which is foreign to the host vector.

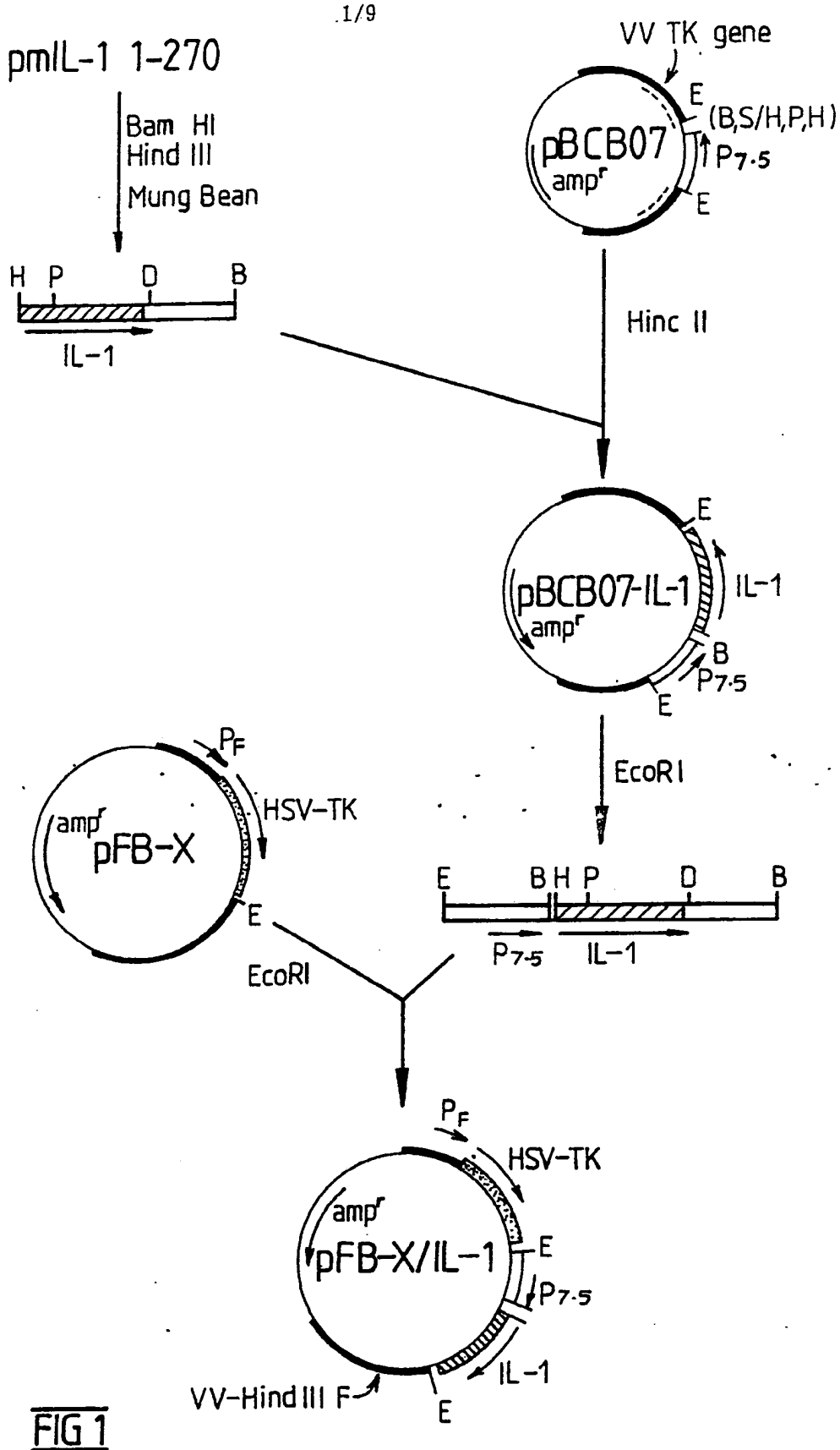
9. Use of a recombinant vaccine according to any one of claims 1 to 6, for the production of an immune response in a human or animal, particularly an immunodeficient or immunosuppressed human or animal.

10. A method for producing an immune response in a human or animal, particularly an immunodeficient or immunosuppressed human or animal, which comprises the step of administering to the human or animal a recombinant vaccine according to any one of claims 1 to 6.

11. A recombinant vaccine for use in the treatment of immunodeficient or immunosuppressed individuals infected with the human immunodeficiency virus (HIV), which comprises a vaccine vector which incorporates a nucleotide sequence capable of being expressed as all or a part of an antigenic polypeptide derived from the human immunodeficiency virus (HIV), together with a second nucleotide sequence capable of being expressed as all or a part of a lymphokine effective in enhancing the immune response of the individual to the HIV antigenic polypeptide.

12. A recombinant vaccine according to claim 11, wherein said vaccine vector is vaccinia virus.

13. A recombinant vaccine according to claim 11 or claim 12, wherein said lymphokine is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4 and γ-interferon.


pmIL-1 1-270
Bam HI
Hind III
Mung Bean
H P D B
IL-1
VV TK gene
E
(B,S/H,P,H)
pBCB07
amp^r
P7·5
E
Hinc II
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amp^r
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B
P7·5
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EcoRI
P_F
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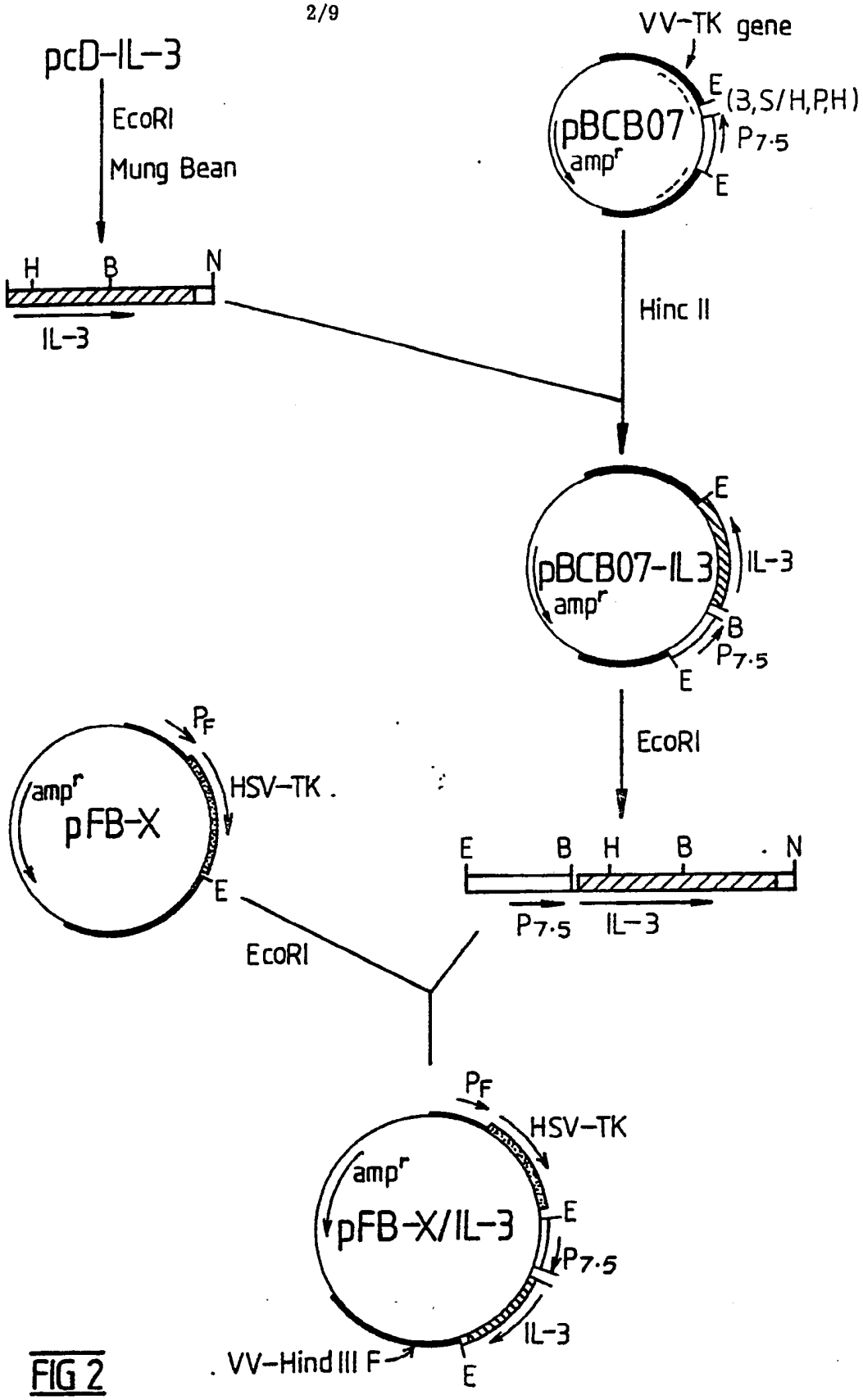
E

FIG 1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Hinc II
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IL-3
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E
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P_F
amp^r
pFB-X
HSV-TK
E
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E

FIG 2

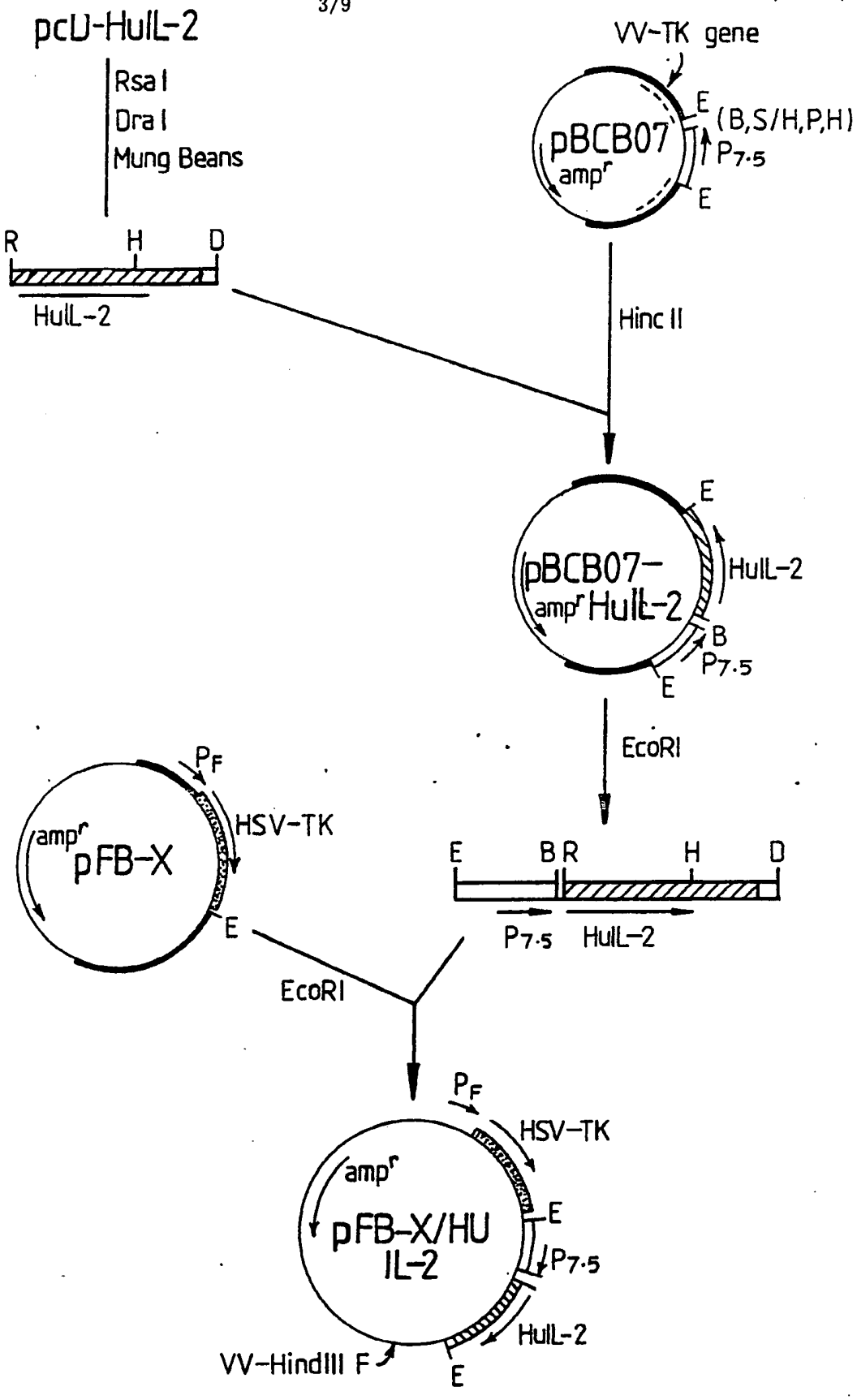


FIG 3

SUBSTITUTE SHEET

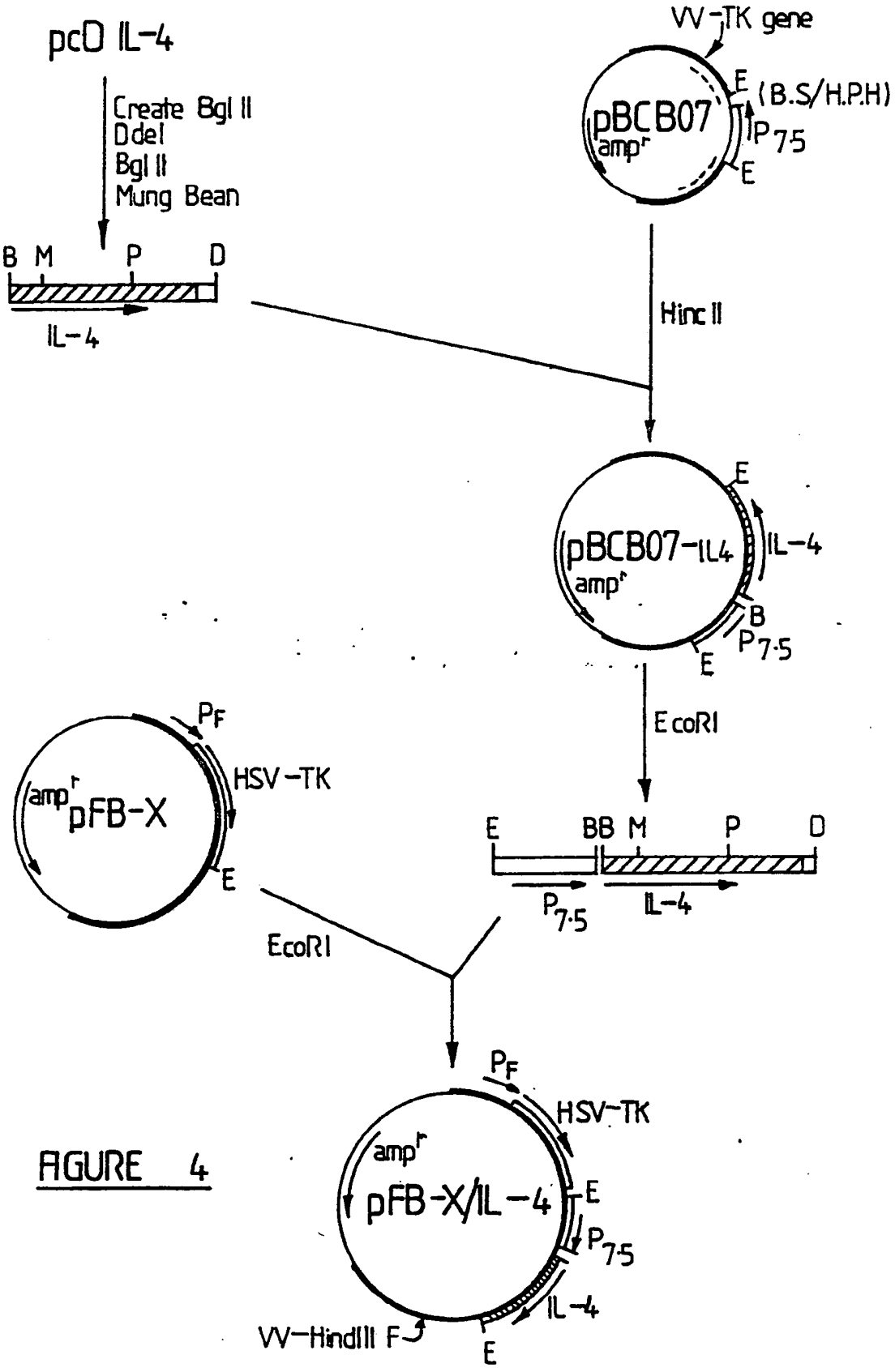

pcD IL-4
Create Bgl II
Ddel
Bgl II
Mung Bean
B M P D
IL-4
VV-TK gene
pBCB07
amp^r
E
(B.S/H.P.H)
P7.5
E
Hinc II
pBCB07-IL4
amp^r
IL-4
B
P7.5
E
EcoRI
E BB M P D
P7.5
IL-4
PF
HSV-TK
amp^r pFB-X
E
EcoRI
PF
HSV-TK
amp^r
pFB-X/IL-4
E
P7.5
IL-4
VV-HindIII F
E

FIGURE 4

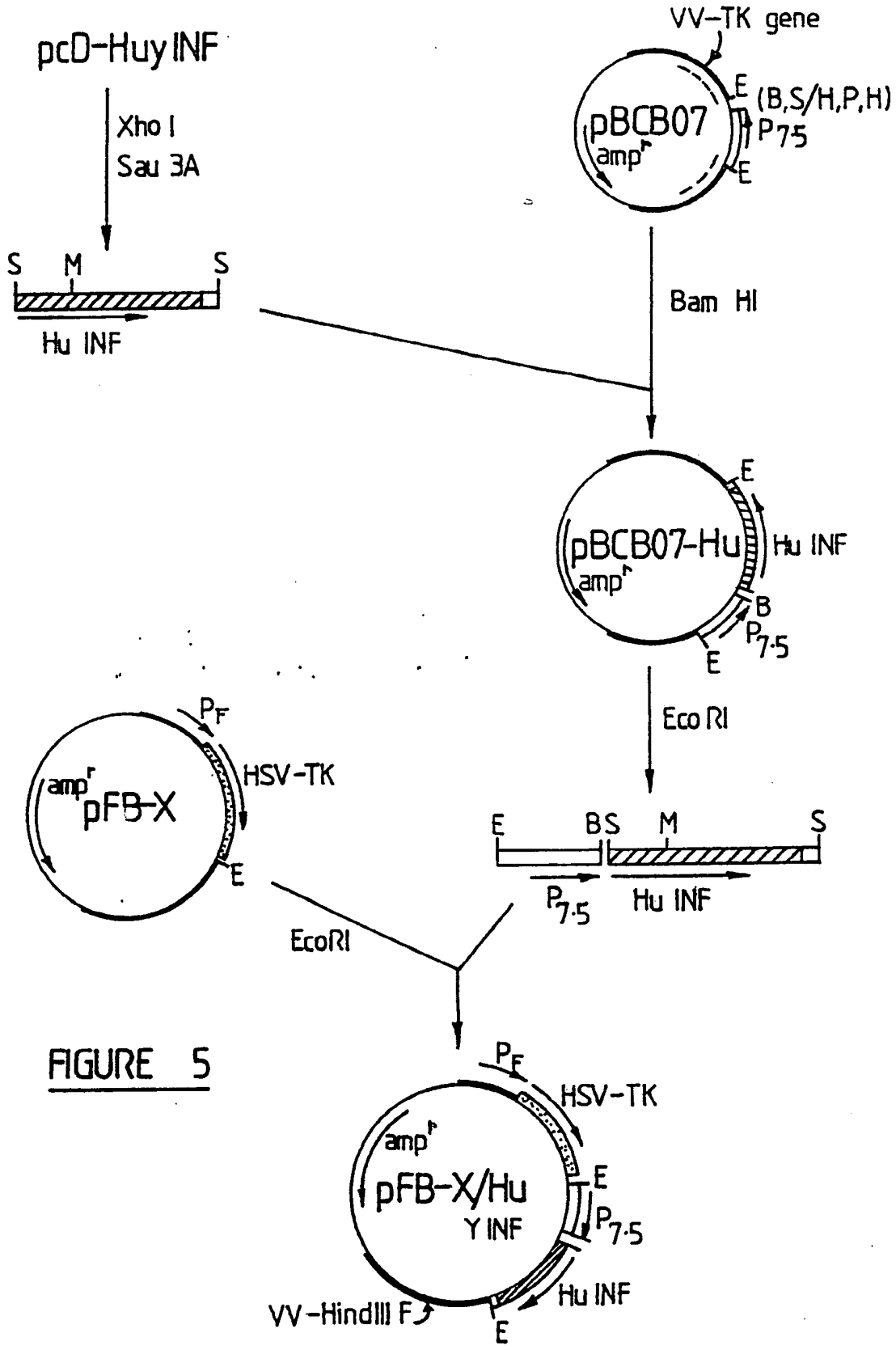

pcD-Huy INF
Xho I
Sau 3A
S M S
Hu INF
VV-TK gene
pBCB07
amp^r
E
(B,S/H,P,H)
P7·5
E
Bam HI
pBCB07-Hu
Hu INF
B
P7·5
E
Eco RI
PF
amp^r
pFB-X
HSV-TK
E
E BS M S
P7·5
Hu INF
EcoRI
PF
HSV-TK
amp^r
pFB-X/Hu
Y INF
E
P7·5
Hu INF
VV-HindIII F
E

FIGURE 5

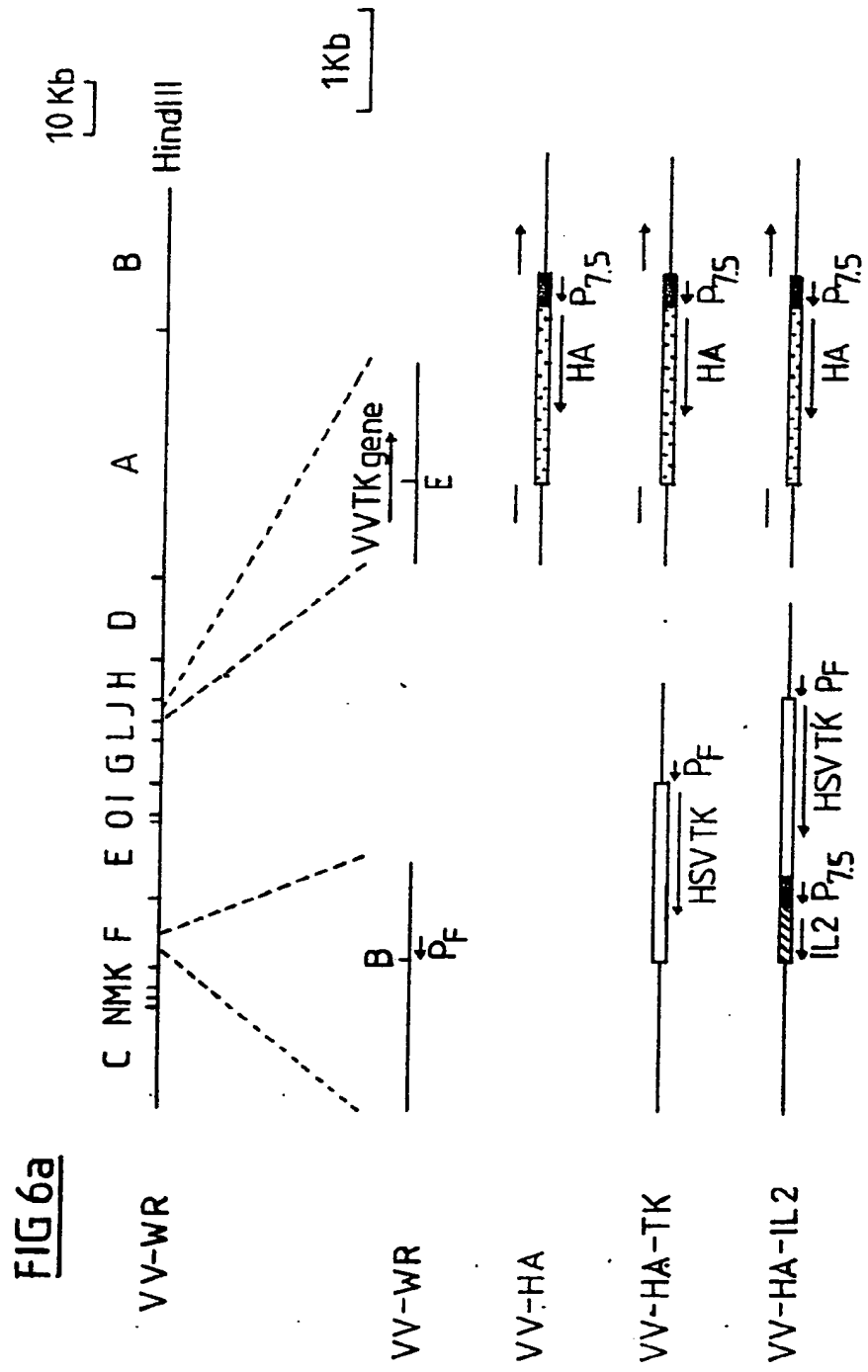

10 Kb
1Kb
HindIII
C NMK F E OI G L J H D A B
VV-WR
VVTK gene
E
B
P_F
HA
$P_{7.5}$
HSV TK
IL2
VV-WR
VV-HA
VV-HA-TK
VV-HA-IL2

FIG 6a

FIG 6b

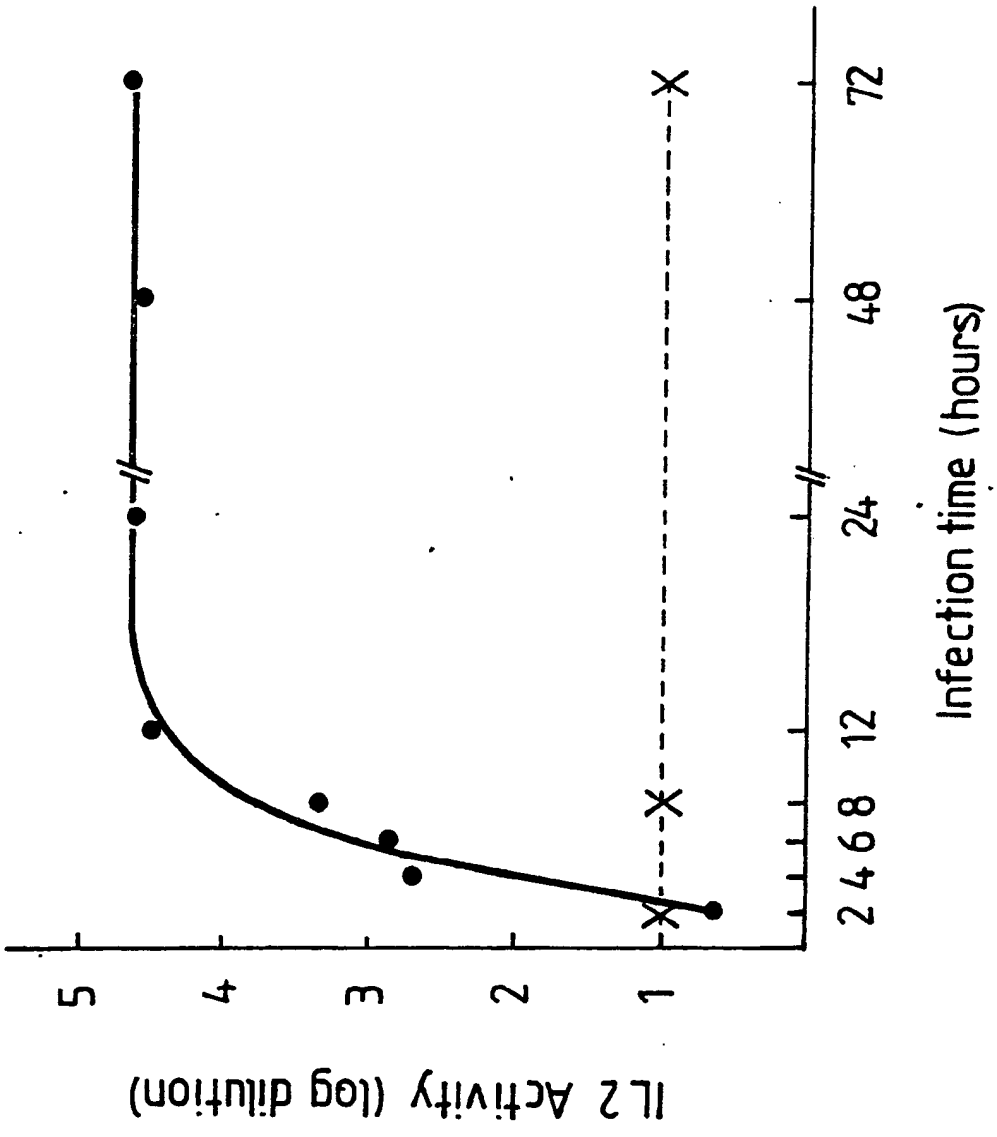
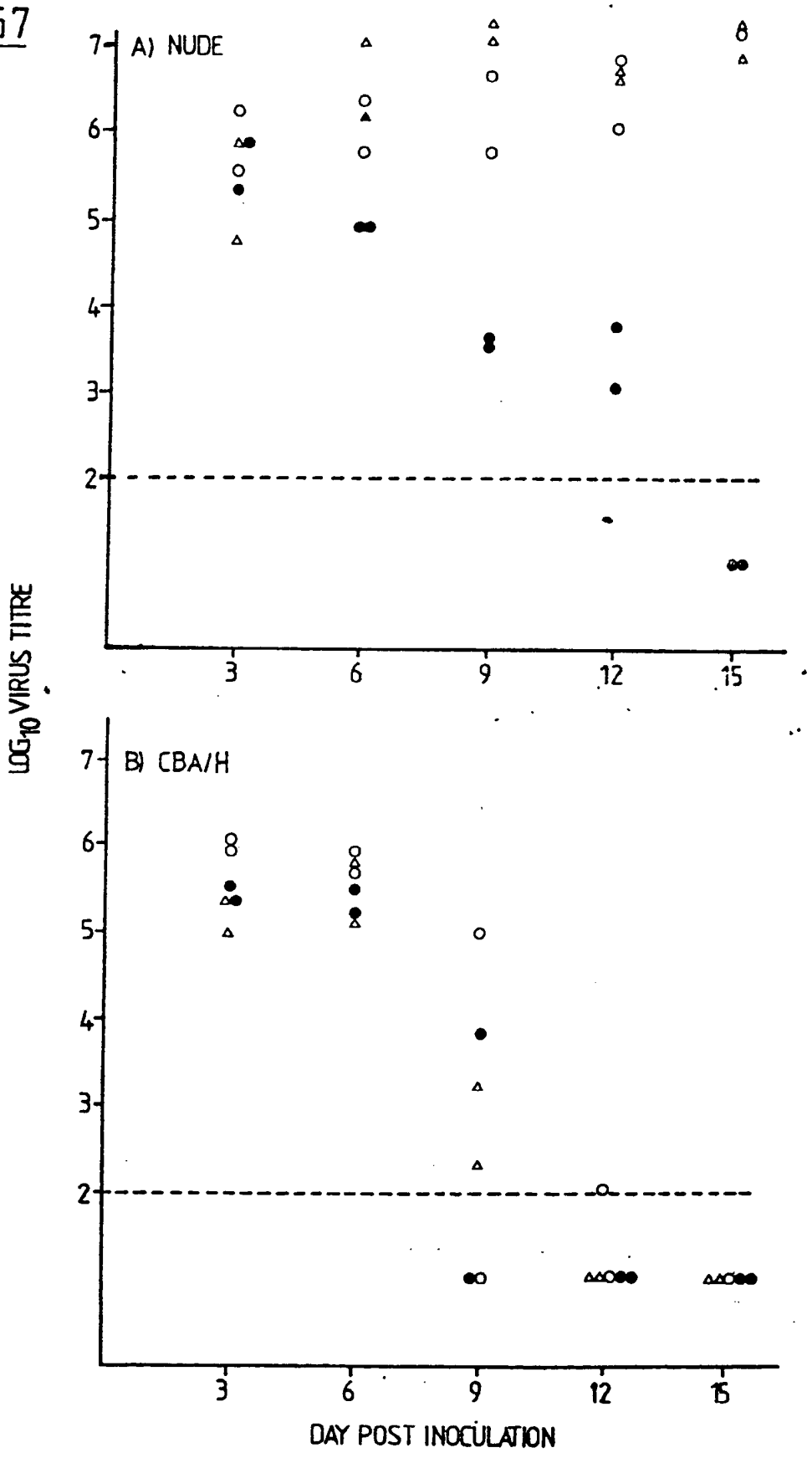

IL 2 Activity (log dilution)
5
4
3
2
1
2 4 6 8 12 24 48 72
Infection time (hours)

FIG7


A) NUDE
B) CBA/H
LOG10 VIRUS TITRE
DAY POST INOCULATION
7
6
5
4
3
2
3
6
9
12
15

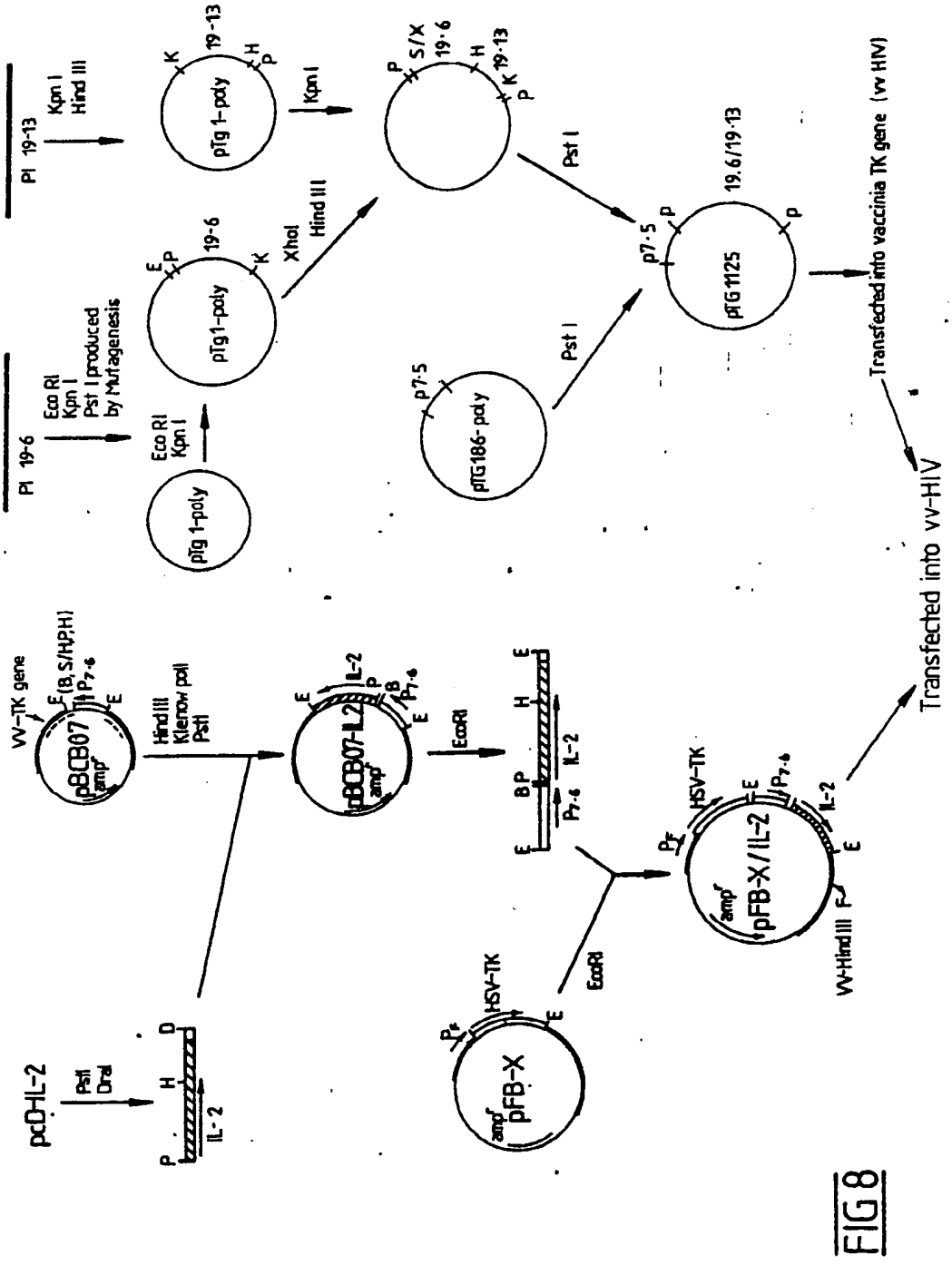


FIG 8

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 87/00246

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C12N 15/00, 7/00, A61K 39/285, 39/21, 39/145, 45/02, 37/02 // C12R 1:91

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC	C12N 15/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

AU : IPC as above
C.A. KEYWORDS (INTERFERON or INTERLEUKIN) and (VACCINIA or ADENOVIRUS)

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	AU,A, 36635/84 (GENENTECH INC.) 20 June 1985 (20.06.85) See page 11	(1-3,6-8)
X,P	EP,A, 206939 (TRANSGENE S.A.) 30 December 1986 (30.12.86) (& AU 59676/86)	(1,3-7)
X,P	EP,A, 206920 (TRANSGENE S.A.) 30 December 1986 (30.12.86) (& AU 59579/86)	(1,3-7)
X,P	AU,A, 59453/86 (KANEGAFUCHI KAGAKU KOGYO KABUSHIKI KAISHA) 8 January 1987 (08.01.87) See page 23 lines 11-19	(1,3,7)
X	AU,A, 11674/83 (BIOGEN N.V.) 1 September 1983 (01.09.83) See page 25 lines 1-23	(1,3,6,7)
X	Proceedings of the National Academy of Science USA, Volume 82, No.3 issued 1985, February (Washington D.C.), R.J. Kaufman, "Identification of the components necessary for adenovirus translational control and their utilization in cDNA expression vectors", see pages 689-693	(1,3,4,6,7)

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 November 1987 (17.11.87)	(30-11-87) 30 NOVEMBER 1987
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	J.H. CHAN

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU **87/00246**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 36635/84	DK 6009/84	EP 146354	FI 844934
	HU 36187	IL 73803	JP 60202899
	NO 845042	PT 79691	ZA 8409767
EP 206920	AU 59579/86	DK 798/87	FR 2583429
	WO 8607609	ZA 8604525	
EP 206939	AU 59676/86	FR 2583770	ZA 8604561
	DK 878/87		
AU 59453/86	EP 207518	JP 62011095	
AU 11674/83	DK 734/83	EP 88540	ES 519953
	IL 67961	JP 59051792	ZA 8301094

END OF ANNEX